OCT 1

BULLETIN

OF THE

Medical Libr

JOHNS HOPKINS HOSPITAL

(THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL)

(SUPPORTED BY THE DE LAMAR FUND OF THE JOHNS HOPKINS UNIVERSITY)

Vol. XLIII, No. 4 OCTOBER, 1928

Contents

The Structure and Function of the Kidney of *Lophius piscatorius*. E. K. MARSHALL, JR., AND ALLAN L. GRAFFLIN..... 205

The Threshold for Electrical Excitation of the Motor Cortex of Anesthetized Mammals. SARAH SHELDON TOWER..... 237

Material from Lymph Nodes. III. Gonococci from Lymphoid Tissue in a Case of Chronic Infectious Arthritis. CLAUDE E. FORKNER, M.D..... 257

PUBLISHED MONTHLY

THE WILLIAMS & WILKINS COMPANY

BALTIMORE, U. S. A.

Made in United States of America

Left: Cod with enlarged Liver

Below: Prime Livers constitute 1/7 the weight of the Cod

Food of the Newfoundland Cod makes Vitamin Potency in Cod Liver Oil

THIS is a typical cod, the liver of which is used in the making of Mead's Standardized Cod Liver Oil—plain and flavored. The belly is enlarged with a huge liver—1/7 the weight of the fish. *One seventh the weight of the fish* from which the oil is rendered in the proportions of 3 gallons of oil from 5 gallons of livers, when the fish are in the best physiological condition.

In Newfoundland, Cod Liver Oil production under the Mead Johnson policy is a leading industry. Codfish, dried, are by-products. Quite the reverse from other fisheries. The Newfoundland Cod after a season of gormandizing on caplin, squid, herring and sea plant life or algae, are in excellent condition for oil production.

Food in abundance—as high as 52 caplin have been found in the stomach of a single cod—is converted into a biologically certain, potent, vitamin-containing cod liver oil.

Biological Assay

Biological assay in the laboratories of Mead Johnson and Company tests the potency of each batch of Mead's Cod Liver Oil. Rats are used. After a rickets-producing diet, advanced healing must be shown in 3 days according to the Shipley line test.

MEAD JOHNSON & COMPANY

Evansville, Indiana

Samples and Literature on Request

Oil on way to market

Caplin

A Squid

Entered as second-class matter at the Baltimore, Md., Postoffice. Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917. Authorized on July 3, 1918.

Copyright, 1928, by The Williams & Wilkins Company

THE STRUCTURE AND FUNCTION OF THE KIDNEY OF LOPHIUS PISCATORIUS[1,2]

E. K. MARSHALL, JR., AND ALLAN L. GRAFFLIN

From the Mt. Desert Island Biological Laboratory, Maine, and the Laboratory of Physiology, The Johns Hopkins University School of Medicine

In 1902, Huot in a short article on the morphology of the Lophobranchs (to which division belong the well-known sea-horse and pipe-fish) described the kidney of these fish as having neither an arterial supply of blood nor glomeruli. A few years later, Audigé (1910), in a general study of the structure of the kidney in Teleostean fish, records the same findings for the kidney of *Lophius piscatorius*[3] (goose fish, monk fish, angler fish). The kidney of this fish had been previously studied by Balfour (1882) and Vincent (1898). Both of these state that glomeruli are present, although the former admits that his specimens were too badly preserved to allow him to be certain. When one considers the great amount of work which has been done on the frog's kidney, and the importance attached to these studies on account of the double blood supply to the tubules, it is quite surprising that little work has been done either to confirm and extend these extremely interesting anatomical observations of Huot and Audigé or to study the function of such a pure tubular kidney.

A functional study of the kidney of *Lophius piscatorius* was commenced in the summer of 1926 and observations were also made in an attempt to confirm the essential findings of Audigé. Owing, however, to certain definite differences in our anatomical findings from those of Audigé, consisting essentially in the presence of an arterial supply and of occasional structures resembling glomeruli, it soon became

[1] This paper was presented at the Ann Arbor meeting of the American Physiological Society (see Amer. Jour. Physiol., 1928, lxxxv, 391).

[2] Received for publication June 14, 1928.

[3] Some observers feel that on this side of the Atlantic we have a different species from that of Europe and propose calling it *Lophius americanus* (see Dahlgren, 1928). However, we have retained the name *Lophius piscatorius*.

evident that a more careful and complete morphological study of the kidney must be made before the physiological observations could be properly interpreted. The present report gives the results of such a morphological study, and also contains such physiological observations as we have at present completed.

ANATOMICAL

Material and methods. The fish used in this investigation were obtained off Mt. Desert Island, Maine. Only freshly killed specimens

FIG. 1. A GOOD-SIZED SPECIMEN OF LOPHIUS PISCATORIUS (LINNÉ), OR LOPHIUS AMERICANUS, ACCORDING TO SOME OBSERVERS

The width of the boards is 5 inches. Photograph by Dr. D. S. Johnson, Salisbury Cove, Maine.

were used to obtain material. In working out the structure of the kidney, most of the well-known methods were employed; namely, maceration, vascular injections, ureteral injections and serial sections of material fixed and stained in various ways.

The maceration technique used by Peter (1909), Huber (1911), Traut (1923), Stewart (1927) and others has proved to be one of the most satisfactory methods for ascertaining the morphology of renal tubules. Some of the kidneys were injected through the ureter with a mixture of ferrocyanide and ferric ammonium sulphate for subsequent precipitation in the tubules as prussian blue. These injections

were only partially successful, and it seemed almost impossible to avoid rupture into the veins. It was found by experience that the best maceration material was obtained by leaving the kidneys in a mixture of equal parts of concentrated hydrochloric acid (sp. gr. 1.18–1.19) and distilled water for 14 hours, after which they were carefully washed free from the acid and placed in distilled water, containing a crystal of thymol. We found it distinctly advantageous to leave the kidneys in distilled water for a fairly long time before attempting to tease the tissues, and invariably a kidney that was very poor teasing material at the end of 48 hours was excellent at the end of a week. The material remained suitable for teasing purposes for fully two months. Very finely pointed phosphor-bronze needles were used in place of glass with good results.

For the gross arterial supply of the kidney, a starch-vermilion-glycerine mass was injected posteriorly through one of the epibranchial arteries, and the relationships studied with the kidneys *in situ*. Subsequently, the kidneys were transferred to 10 per cent formalin, and later cleared by the Spalteholz method, after which free-hand sections were made with a razor and studied under the Greenough binocular dissection microscope.

Complete vascular injections were obtained by using India ink. Preparatory to making these injections, isotonic salt solution, to which had been added amyl nitrite, was injected posteriorly through one of the epibranchial arteries until the venous return through the sectioned posterior cardinal veins was quite clear. The salt was then followed with 50 per cent India ink in sufficient quantities to give a good black venous return. The kidneys were subsequently fixed in formalin for clearing by the Spalteholz method and in Bouin's and Zenker's fluids for histological preparations.

Finally, purely arterial injections with India ink were made by following the same procedure as above with the exception that all of the afferent veins to the kidney, including several small channels which are always present, were first doubly ligated and sectioned between the ligatures.

Casts of the Wolffian-duct tree were prepared by injecting Tandler's cold fluid gelatin mass, colored with vermilion, under 6 inches' gravity pressure, for 1 to 2 minutes. The kidney was then fixed *in toto* in

large quantities of 10 per cent formalin and cleared by the Spalteholz method.

The general structural characteristics of the kidney were studied in

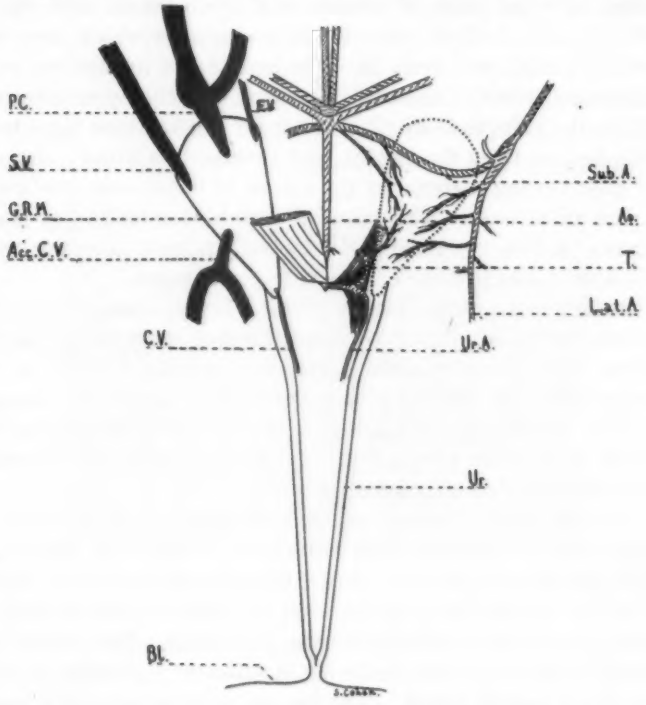


FIG. 2. A DRAWING OF THE KIDNEYS IN SITU, SHOWING THE GROSS ANATOMICAL ARRANGEMENT

The arterial branches to the kidney were distended with injection mass, and the drawing gives an exaggerated idea of their size. The short single ureteral duct is characteristic of the female.

P.C., posterior cardinal vein
E.V., epibranchial vein
S.V., subclavian vein
G.R.M., gill-raising muscle
Acc.C.V., accessory caudal vein
C.V.,caudal vein
Sub.A., subclavian artery
Ao., dorsal aorta
T., tendon
Lat.A., lateral artery
Ur.A., ureteral artery
Ur., ureter—Wolffian duct
Bl., bladder

a complete set of serial sections at 50μ of a kidney taken from a good-sized specimen of *Lophius*. Many other serials of selected blocks were prepared for detailed histological study.

Gross anatomy. Balfour (1882) and Audigé (1910) have both given a description of the gross anatomy of the kidneys of *Lophius piscatorius*, and it will be sufficient here to give a brief summary of the relationships as we have found them in American specimens. The kidneys are two flattened, pear-shaped masses, retroperitoneal in position, displaced anteriorly so that they lie on the brachial plexus, and separated from the vertebral column on each side by the genital fossa, which is completely covered in most instances by the gill-raising muscle. Arising from the tapering posterior end of the kidney is the Wolffian duct which runs caudally to fuse with its fellow from the opposite side in the mid-line before entering the bladder. This fusion usually takes place close to the bladder in the female and farther away from the bladder in the male.

The blood supply of the kidney (fig. 2) is almost entirely venous, but possesses a small arterial component which was not described by Audigé. The afferent venous trunks are the caudal vein (C.V.) which courses medial to the Wolffian duct and enters the posterior end of the kidney; the caudal accessory vein (Acc.C.V.) which is larger than the preceding and courses deep through the back musculature to enter the kidney on the lateral border; the subclavian vein (S.V.) which empties into the kidney at the antero-lateral angle; and the epibranchial vein (E.V.) which enters at the antero-medial angle. In addition to these, there are usually one or two small venous trunks which reach the anterior border of the kidney lateral to the posterior cardinal vein, and sometimes one or more fine channels which may enter either the lateral or dorsal surface of the kidney. The whole kidney can be injected from any one of the afferent veins. The large posterior cardinal vein (P.C.) is the only efferent trunk from the kidney. It is evident that definite renal arteries are absent and that the main arterial supply to the kidney is from minute branches of the lateral and ureteral arteries. In addition, there are usually two or three fine branches from the subclavian artery and from the region of the arterial *carrefour*. These arterial twigs have been shown to supply the "glomerular tufts" and their sinusoidal tissue, the larger venous

channels, as vasa vasorum, and the Wolffian duct and its larger branches. In addition, small arterial channels can be seen to break up into the renal tissue itself.

The only other striking feature of the kidney in the gross is the presence of small, round, white bodies partly embedded in the kidney substance. These are discussed at length below.

Serial sections of an entire kidney. An entire kidney, approximately 6 x 3 cm., was serially sectioned at 50μ, and each of the sections was carefully studied under the Greenough binocular at a magnification of 40. The most important fact yielded by this study was that in this kidney here and there were seen capillary tufts, closely resembling glomeruli, but which, for reasons which will be given below, we have termed pseudoglomeruli. These tufts were nearly always just beneath the renal capsule and always occurred in association with a peculiar sort of tissue, very vascular in nature, called by us sinusoidal tissue, a tuft of which contained usually one, but sometimes two pseudoglomeruli. In the whole kidney there were only 78 of these structures.

The study showed further that there was a practically uniform distribution of lymphoid or pseudolymphoid tissue and tubular substance throughout the kidney. The lymphoid tissue occurs between the tubules and has been described at length for the kidneys of many fish (Audigé, 1910).

Two accessory structures of the kidney were located in this serial. Encircling the posterior cardinal vein on its ventral aspect is a fairly large, nonencapsulated mass of adrenal tissue, which may be seen in thin sections of material fixed in Orth's fluid to be made up of a mixture of interrenal and chromaffin cells (Plate 3, Fig. 14). In addition, the sections showed five spherical bodies, four of them peripheral and one central in position, all definitely encapsulated, and all staining deep blue (H. and E. sections). Four peripheral bodies of this type were subsequently found in all *Lophius* kidneys as round, white bodies. In thin section (Plate 3, Fig. 13), they present a very odd appearance.

Finally, many groups of large nerve cells, resembling sympathetic ganglion cells, were identified on the surface of the kidney. Subsequent study of thinner sections revealed numerous nerve trunks and

similar large cells in the substance of the kidney. These cells are well shown in Plate 1, Figure 9.

The renal tubules. This kidney is made up of great numbers of irregularly convoluted tubules, with perfectly definite blind endings. The length of the tubules is about 10 mm. in macerated material and the average diameter is 50μ in sections.

A large number of terminal uriniferous tubules were carefully teased from their connection with a collecting duct to their termination as a blind ending. That the morphological character of these endings is variable can be appreciated from those pictured in Figure 5, all of

FIG. 3. DESCRIPTION IN TEXT
Drawn with camera lucida at about 50×. Reduced ½

FIG. 4. DESCRIPTION IN TEXT
Drawn with camera lucida at about 50×. Reduced ½

which were isolated from a relatively small portion of the same kidney. Endings similar to the one marked * in the figure are undoubtedly the predominant type encountered in maceration. Furthermore, Sudan III demonstrates an abundance of fat droplets in the tubular epithelium, which invariably disappear as the ending is approached. Systematic teasing of representative portions of the kidney show that there is a fairly uniform distribution of tubular endings throughout. Furthermore, analysis of the individual isolated tubules demonstrates a total lack of any uniformity in the location of convolutions or straight portions, so that there is no question of a proximal and distal convolution or a loop of Henle, which characterize the renal tubules of many other forms (Figs. 3, 4, 6). The lack of differentia-

tion in the morphology of the tubules is in keeping with an essentially uniform tubular epithelium.

The epithelial cells of the tubules are cuboidal in shape, with a coarsely granular cytoplasm, an indistinct cell outline and a spherical

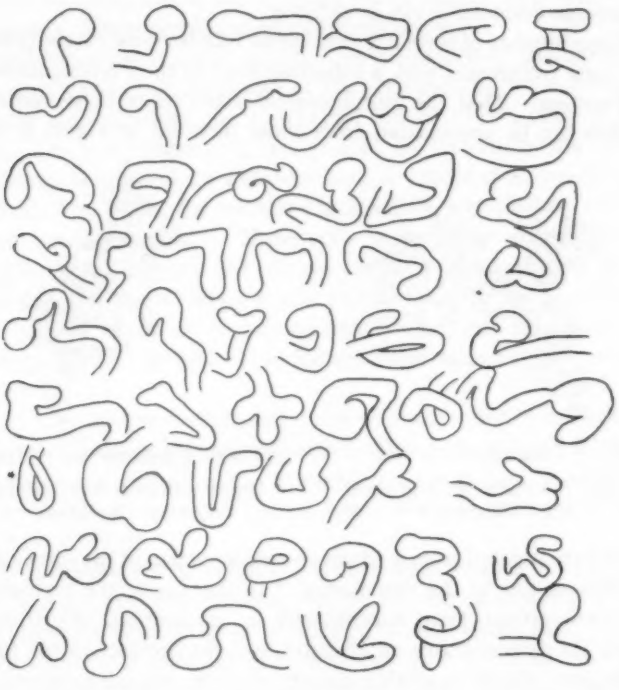


FIG. 5. FIFTY-FOUR BLIND TUBULAR ENDINGS, SHOWING THE VARIOUS FORMS ENCOUNTERED IN MACERATION MATERIAL

All of the endings were teased from a relatively small portion of kidney substance.

or oval nucleus. The nuclear membrane stands out distinctly, but the body of the nucleus is pale and vesicular, with the exception of a radiating or spherical nucleolar condensation which takes the stain very deeply. The tubular cells lie on a distinct and rather heavy

basement membrane. Careful study of our sections yields no essential difference between the tubular epithelial cells at any point, other, perhaps, than a small degree of flattening as the caliber of the tubule diminishes toward the end. Histological study of kidneys with complete vascular injections with India ink demonstrates wide and freely anastomosing venous channels closely investing the outside of the tubules (Plate 1, Fig. 8). Moreover, this arrangement can be readily appreciated in the uninjected kidneys, in which definite endothelial nuclei are evident along the outer edge of the basement membrane. In sections picked at random, the external diameter of 125 tubules was accurately measured, and the average of these measurements was 50μ.

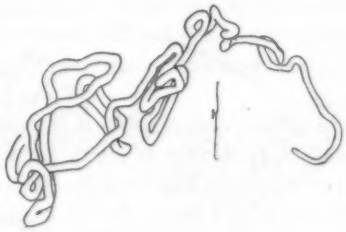


FIG. 6. A TERMINAL URINIFEROUS TUBULE DRAWN WITH THE CAMERA LUCIDA
A scale is given for comparison. Total length of scale is 0.5 mm.

Wolffian duct and collecting ducts. The Wolffian duct, arising from the tapering posterior end of the compact kidney, is extremely interesting. There is no renal pelvis, but the duct, once inside the renal substance, forms a flat, serpentine tube which ascends and curves from side to side through the center of the kidney. Hence a considerable thickness of a Spalteholz-cleared preparation can be shaved off the ventral and dorsal aspects of the kidney, leaving the complete duct practically unharmed. The diagram (Fig. 7) was drawn from an injected kidney so prepared, and, for the sake of clearness, no attempt has been made to include all of the many fine branches of the main trunk. The epithelial lining of the main Wolffian duct in the kidney and its large branches is tall columnar in nature but becomes flatter as the smaller ducts are approached.

Careful study of teased and of histological material shows that there is the utmost variability in the course, length and size of the collecting ducts, and two of the conditions commonly found in maceration are represented in the camera lucida sketches. Figure 3 shows a fairly large branch of the tree giving off smaller ducts and tubules, with the greatest irregularity as it courses through the tissues, and it will be noticed that several terminal tubules empty independently into a large collecting duct, which also receives a tubule and a fair-sized duct at the same level. Figure 4 shows a small collecting duct scarcely larger than a renal tubule and arising by the union of two uriniferous tubules. These finer ducts often course for long distances through the tissue, with no or only occasional tubular branches, before

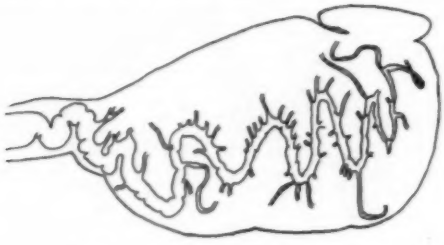


FIG. 7. WOLFFIAN-DUCT TREE

The kidney was injected through the Wolffian duct with gelatin and cleared by the Spalteholz method.

finally splitting into two or three terminal tubules. The small collecting ducts, as seen in section, have in general a greater caliber than the terminal tubules and can be differentiated by their flatter epithelium and the lack of definite granulation in the cytoplasm. The nucleus is similar in staining properties to that of the tubular epithelium.

The great irregularity in the branching of the Wolffian-duct tree, and the frequency with which terminal uriniferous tubules empty directly into the main duct or one of the large branches, leave no alternative but to consider the terminal tubule as the structural unit of this kidney.

Pseudoglomeruli and sinusoidal tissue. In studying the pseudo-

glomeruli we have relied principally on histological sections of injected and uninjected material. Sections of injected material show at a glance the extreme vascularity of the sinusoidal tissue and also show the capillary tuft of the pseudoglomerulus to be well injected (Plate 3, Fig. 12). This knowledge was subsequently made use of in picking out the pseudoglomeruli and their accompanying sinusoidal tissue in injected material which had been fixed. It was found, on searching the surface of the kidney under the low power of the Greenough binocular, that in the purely arterial injections the tufts of sinusoidal tissue, with their pseudoglomeruli, are supplied directly by arterial twigs, so that they stand out as black areas just beneath the capsule of the kidney. In this way we were able to select a glomerulus almost at will. On the other hand, in uninjected kidneys there is absolutely no way of locating the capillary tufts in the gross, so that we were forced to cut hundreds of sections, selecting blocks from the periphery of the kidney.

The great rarity of the pseudoglomeruli made the task of searching for them in macerated material extremely tedious. In systematic teasing of the periphery of a well-macerated kidney only one of these structures was found, although about 300 blind tubular endings were isolated from the same kidney. The afferent and efferent vessels, one larger than the other, could be readily identified, but the Bowman's capsule, although irregular in shape, was perfectly intact so far as we could determine.

Histologically, the pseudoglomerulus is a large capillary tuft (Plate 1, Fig. 9), usually markedly irregular in shape, with an afferent and efferent vessel and invariably showing quite a wide space between the tuft and the Bowman's capsule. The tuft shows great variability in its size, sometimes being very large, at other times consisting of only several small capillaries, so that it looks almost atrophic. The tuft is closely invested by a single layer of epithelial cells which are quite flat except in the region of the nucleus, which causes a distinct bulge. The nucleus is similar to that of the tubular epithelium and the cytoplasm is finely granular. Bowman's capsule is lined with very flat epithelial cells having slender, elongated nuclei, so that they are in all respects similar to endothelial cells.

The sinusoidal tissue is made up of anastomosing cords of cells,

(Plate 1, Fig. 9), rather large, usually oval or pear-shaped, with the nucleus close to one surface. The cytoplasm is homogeneous, showing no granulation and never takes the eosin as brightly as the tubular cells. The cells are rather sharply outlined. The nucleus is spherical or oval in shape; the nuclear membrane is distinct, but the body of the nucleus is rather pale and vesicular, with small condensations of chromatin material scattered irregularly throughout. Closely applied to the cords of cells are flat endothelial cells, lining the sinusoidal channels which occupy the interstices of the parenchyma. In macerated material sinusoidal tissue looks like a mass of coiled blood vessels.

As was said above, the vascular bed of the sinusoidal tissue and glomerular tufts was completely filled in kidneys injected arterially after the venous return had been cut off. Careful histological examination demonstrates an arterial branch supplying the tissues in each instance. Even when the tuft and the sinusoidal tissue are beneath the surface of the kidney, an arterial connection can be demonstrated. This relationship is shown in Plate 3, Figure 12. On developmental grounds one could hardly conceive of such a glomerular tuft that was not arterially supplied.

Careful study of many 10μ serials had consistently failed to show any connection between Bowman's capsule and a terminal uriniferous tubule or a collecting duct. The capsules were often extremely irregular in shape and sometimes presented diverticula, any one of which, in a single section, might give the impression of the beginning of a tubular connection, but study of adjacent sections was always sufficient to establish the true identity of these outpouchings. Furthermore, the capillary tuft often showed at several places cellular bridges connecting it to the capsule (Plate 2, Fig. 11). In the above serials, however, it was evident that in some cases a section or two had been lost. The importance of determining the presence or absence of a tubular connection led one of us (G) to cut, mount and stain what he knows to be perfect serial sections of six of these pseudoglomeruli, four at 20μ and two at 7.5μ.

Both of the pseudoglomeruli in the 7.5μ serials were carefully traced under the high power, but it was impossible to demonstrate a connection with a functional tubule. Nothing, certainly, could be

more suggestive of a connection than the section seen in the photograph, yet the tubular outpouching ends blindly, so far as we can determine. Significant in the case of this pseudoglomerulus are the facts that the glomerular tuft is at about its maximal size, as seen in the photograph (Plate 2, Fig. 10), and that the capsular space and the tubular outpouching are filled with coagulated fluid. The other pseudoglomerulus likewise possesses an extremely irregular Bowman's capsule and also contains coagulated fluid, but here again it is impossible to demonstrate a connection with a functional tubule. All of the tubular outpouchings seem to end blindly.

In the other serials at 20μ, however, the findings are, for the most part, quite different. One of the tufts, which is so atrophic that it has practically lost its glomerular nature, has a small tubular outpouching which seems definitely to end blindly. The capsule is filled with coagulated fluid. A second tuft has a definite coiled tubular connection which may or may not connect with a functional tubule, this being doubtful because of the thickness of the sections. Here, also, the capsule and tubule are filled with coagulated fluid. A third tuft connects with a fairly long, coiled tubule which seems definitely to connect with a functional tubule in its course. The capsule in this case shows only the barest trace of coagulated fluid in some sections, and the tuft is very well formed with no connecting cellular bridges at odd places to the capsule such as we have found in other tufts. A fourth tuft, located in from the periphery but still getting an arterial supply, shows a perfectly definite tubular connection, whose basophilic, flat epithelium, as it leaves the capsule, shows a gradual transition to an eosinophilic, cuboidal epithelium which is indistinguishable from that of surrounding tubules. The capsule shows a small amount of coagulated fluid and the tuft is very well formed.

In summary, then, we have found one glomerulus which definitely connects with what is, so far as we can determine, a functional renal tubule, and another tuft with which a functional tubular connection is quite likely. On the other hand, we have examined four others with definite tubular outpouchings, which, in all but one doubtful case, seem to end blindly. It is obviously dangerous to draw a negative conclusion from 20μ sections, but the two glomeruli which were cut at 7.5μ point clearly to the lack of a functional connection in at least some cases.

Discussion. Our anatomical findings differ in certain important respects from those reported by Audigé. A definite, although small, arterial supply to the kidney has been conclusively demonstrated and sections have revealed rare structures resembling glomeruli, some of which, at least, seem to be non-functional. The curious sinusoidal tissue, which we described as occurring in connection with the pseudoglomeruli, has not, so far as we can discover, been previously reported. Audigé is very insistent that the anterior third of the kidney consists entirely of lymphoid tissue containing no uriniferous tubules. We have been unable to discover any such region on examination of a large number of specimens; there is a practically uniform distribution of tubules and lymphoid tissue throughout the entire kidney. After insisting on a total absence of glomeruli, Audigé proceeds to describe "*pelotons*," formed by the coiled ends of occasional tubules, which project into the interior of the capillaries or the venous sinuses of the kidney. He points out the superficial resemblance of these structures to glomeruli and suggests that these structures were mistaken by Balfour for malpighian corpuscles. We have searched our complete set of 50μ serial sections as well as our thin series but have never succeeded in identifying these "*pelotons*." Finally, we find an average external tubular diameter of 50μ compared with the average of 6 to 8μ reported by Audigé.

It is difficult to explain these marked discrepancies between our findings and those of Audigé. In view of the fact that the American form is considered by many to be a different species from the European, the simplest explanation would appear to be that there is a species difference in the kidneys. However, we have obtained the kidneys from two specimens of *Lophius piscatorius* caught at Plymouth, England, in order to investigate this matter. The kidneys were fixed in Bouin's fluid and subsequently sections were made from the anterior, middle and posterior portions of the kidney. We can state definitely that uriniferous tubules are distributed throughout the entire kidney; no anterior region of purely lymphoid tissue was found, and in this we confirm Balfour (1882) and Vincent (1898). Furthermore, occasional tufts of sinusoidal tissue with pseudoglomeruli do occur, and are supplied by arterial branches. Finally, nothing resembling Audigé's "*peloton*" was found in over 600 sections. From

our study of these sections we feel that it is quite possible that some species differences in the kidney may exist between the two forms, but do not wish to discuss it on the basis of such limited material of the English form. The general structure of the English specimens, thus, does not accord with Audigé's description and he may have been dealing with another species occurring in the Mediterranean.

Although it appears quite certain that the few pseudoglomeruli which we have described can play no important part in the formation of urine, it is best for the interpretation of physiological findings to assume that all pseudoglomeruli may connect with functional tubules. It is, therefore, important to attempt to compare the number of these structures with the total number of tubules in a kidney. An estimate was made by drawing an entire kidney approximately to scale, and drawing, likewise to scale, a small portion which was selected for teasing. This piece was then teased exhaustively for endings. Subsequently, the size of the small portion as compared with the whole kidney was carefully estimated by several individuals, with as much mathematical accuracy as possible and with ample provision for the larger veins and ducts. Then, assuming a random distribution of tubule endings and so of tubules throughout the kidney, which we had already demonstrated, the total number of tubules per kidney could be approximately determined. The estimates ranged from 168,000 to 215,000. This method is obviously open to great error. The number of tubules was estimated in another way by determining the volume of a single tubule on the basis of the greatest length and the average external diameter (50μ) and then dividing this volume into the total volume of tubular substance of the serially sectioned kidney. Since the greatest length of tubules ever encountered in macerated material was 12 mm., and since the kidneys swell in distilled water, it seemed that 10 mm. could well be used in this calculation. It is evident from the photographs that 40 per cent is a quite conservative estimate of the proportion of tubular substance to the whole kidney. This method gave 180,000 as the total, and we feel that 150,000 tubules, as a minimum for the serial sectioned kidney, is a conservative figure. In view of the fact that there were found 78 pseudoglomeruli in this kidney and since it is quite definite that some of them at least do not connect with renal

tubules, we have one pseudoglomerulus to a minimum of 2000 tubules. The preponderance is sufficiently great to make it evident that physiologically the kidney of *Lophius* may be considered as a practically pure tubular organ and that here we are dealing with tubular excretion of all the substances in the urine.

PHYSIOLOGICAL

Methods. The specimens of *Lophius* used for investigation were kept tied to the laboratory dock in rather deep water. They survived here for several days and frequently for a period of a week. They varied from about 7 to 15 kgm. in weight.

Urine was obtained by catheter from the bladder, and blood was drawn with a syringe and needle by puncturing the heart in the intact animal. It was found to be comparatively simple to obtain several specimens of blood and urine from the same fish in a period of several hours or on successive days. The fish were out of the water only for the few minutes necessary to collect the samples of blood or urine.

Serum or oxalated plasma was employed in all the blood analyses. The methods of analysis employed for blood and urine were: chloride' Van Slyke (1923); sulphate, benzidine method (Rosenheim and Drummond, 1914); phosphate, Briggs' modification of Bell-Doisy method (Briggs, 1922, 1924); sodium, Kramer and Gittleman (1924); potassium, Kramer and Tisdall (1921); calcium, Clark and Collip (1925); magnesium, Denis (1922); urea by urease and estimation of carbon dioxide formed (Van Slyke, 1925); uric acid, Benedict (1925); and creatinine and creatine, Folin (1904).

Results. Burian (1909) reports that 2 to 3 kilo specimens of *Lophius* yield 40 to 60 cc. of urine in 24 hours. We have obtained 700 cc. of urine from a large specimen in 22 hours, from another 500 cc. in 19 hours and frequently found the excretion rate per hour to be 10 to 25 cc. It seems evident, therefore, that the kidneys excrete water readily. The urine, whenever tested, has never contained protein or sugar.

The change in composition of the plasma and urine, which is effected by the kidney, as well as a general survey of the composition of the urine and blood serum of this animal, is given in Table I. The

analyses were made on specimens of blood and urine collected simultaneously from the same fish.

If one examines the above analysis of plasma and urine, certain definite differences appear between the composition of this urine and that of mammalian and amphibian urine. The large amount of base eliminated in the urine as magnesium, the minute amount of urea or uric acid in either plasma or urine, and the large amount of creatine in the urine are the most prominent variations.

The only previous investigation, which we can find, on the composition of the blood or urine of *Lophius* is that of Denis (1913). She

TABLE I

Analysis of blood serum and urine of Lophius

	SERUM	URINE
	millimols per liter	*millimols per liter*
Total CO_2	7.1	1.1
Cl	191	226
SO_4	1.2	43.8
PO_4	6.4	1.5
Na	201	117
K	12.1	2.8
Ca	3.5	11.8
Mg	1.0	95.4
Urea	0.5	0.5
Creatinine		0.1
Uric acid	0.05	0.1
Creatine	0.05	2.0

The pH of the serum was 7.3, that of the urine, 6.8.

gives the analysis of one specimen of blood for non-protein nitrogen, urea, ammonia, uric acid and creatine + creatinine. She found 5.7 millimols of urea per liter, somewhat more creatine than the above table indicates, and about the same amount of uric acid. An analysis is also reported for a composite sample of urine from six goosefish. The total nitrogen, urea, ammonia, uric acid, creatinine and creatine as well as the phosphates, chlorides, sulphates and total sulphur were estimated. The creatine comprised 16.6 per cent of the total nitrogen, while the urea amounted to over 8 millimols per liter, comprising 14.4 per cent of the total nitrogen. We have never been

able to find more than a trace of urea in either the blood or the urine of this fish and can only ascribe Denis' findings to the use of a non-specific method for the hydrolysis and estimation of urea.

The very small amounts of urea, uric acid and creatinine found in the urine raise for consideration the question of the form in which the end product of protein metabolism is eliminated Urea or uric acid are the only two forms in which the greater portion of the nitrogen is eliminated in vertebrates. Although no accurate determinations were made, special tests indicated that no appreciable amount of nitrogen is eliminated as ammonia or amino-acids. Four specimens of urine from different fish in which the creatine and creatinine had been estimated were preserved with sulphuric acid and later analyzed for their total nitrogen content. In Table II the results are given.

TABLE II

Total nitrogen and creatine of Lophius' urine

SAMPLE NUMBER	TOTAL N	CREATINE N	PER CENT OF TOTAL-N
	mgm. per cent	*mgm. per cent*	
1	33	10.7	32
2	80	17.9	23
3	57.6	30.7	53
4	52.8	29.6	56

It is evident that a large percentage of the nitrogen of the urine is not accounted for by known consitutents. The very small percentage of total nitrogen in the urine means that only a few tenths of a gram of nitrogen is excreted per day. This is somewhat difficult to reconcile with the fact that this fish is a voracious feeder on protein. A whole goose, a dozen flounders, or a haddock over two-thirds the length of the *Lophius* may be taken at a meal (Dahlgren, 1928). The explanation would seem to be either that nitrogenous waste products are eliminated by some other channel than the kidney or that meals are taken very infrequently and digestion and absorption are extremely slow. The latter possibility appears to us to be the more reasonable.

Tables III and IV give the data from several experiments in which analyses of plasma and urine were made. It is evident from these tables that the chloride concentration in the urine is always slightly

higher than that in the blood; that urea occurs in only small amounts in both urine and blood and is never more concentrated in urine than in blood; that sulphate and creatine are highly concentrated in their

TABLE III

Analysis of plasma and urine of Lophius

		PLASMA	URINE
		millimols per liter	*millimols per liter*
Lophius II August 12	Cl	166	202
	PO_4	5.1	14.2
	Urea	0.4	0.3
Lophius III August 13	Cl	154	177
	PO_4	4.5	18.3
	Urea	0.5	0.3
August 14	Cl	160	184
	PO_4	3.7	14.0
Lophius IV August 26	Cl	228	229
	PO_4	3.6	7.3
	SO_4	Trace	62
	Urea	0.6	0.3
	Creatinine		0.3
	Creatine	Trace	7.3
Lophius V August 27	Cl	168	199
	PO_4	5.3	7.7
	SO_4		180
	Urea	0.7	0.4
Lophius VIII September 1	Cl	200	219
	SO_4	3.5	55
	PO_4	6.4	0.1
	Urea	0.3	0.2
	Creatine	0.3	18

passage through the kidney; and that phosphate may be present in the urine in greater or in lesser concentration than in the blood.

In order to find any differences in the function of the kidney of *Lophius* due to its peculiar structure, it is unfair to compare it with that of the mammal or even of the frog, but comparison should be

made with the function of the kidneys of other fish which possess glomerular kidneys. Unfortunately, very little is known of the function-

TABLE IV

	TIME	PLASMA		URINE		
		Millimols per liter		Cubic centimeter	Millimols per liter	
		Cl	PO_4		Cl	PO_4
Lophius:						
August 27	9:40 a.m.	169	5.3	92	199	7.7
	2:50 p.m.			32	214	6.7
	3:50 p.m.	175	5.9	8	222	6.0
	4:50 p.m.			1.5		5.7
August 28	10:00 a.m.			39	238	3.4
	10:45 a.m.	180	5.7	21	237	2.8

TABLE V

Analysis of blood and urine of cod

	SERUM	URINE
SO_4	Trace*	117.2
PO_4	5.7	14.4
Urea	1.5	1.6

* Less than 0.5 millimol.

TABLE VI

Analysis of blood and urine of skate

	PLASMA	URINE
Cl	230	229
SO_4	Trace*	44.3
PO_4	0.7	11.4
Mg	2.4	65
Urea	382	47
Creatine		3

* Less than 0.5 millimol.

ing of fish kidneys and it seems impossible even to find simultaneous analyses of blood and urine. The following incomplete analyses of

blood and urine taken simultaneously from the heart and urinary bladder of two fish, a cod (*Gadus callarias*) and a female skate (*Raia stabulaforis*), are given in Tables V and VI. The kidneys of both of these fish show on section numerous glomeruli with which tubular connections can be demonstrated. All concentrations are expressed in millimols per liter.

Phenol red has been extensively used in investigations on renal function in both the frog and mammal. It is of great importance to determine its behavior in the kidney of *Lophius*. The two protocols given below indicate that it is excreted by this kidney and may be present in the urine in much greater concentration than in the blood. In the first experiment an intraperitoneal injection was made but the extreme slowness of absorption from the peritoneal cavity as compared with that in the the mammal prevented its appearance in the blood or urine in a high concentration. In the second experiment an intracardiac injection was made; but since no proof exists that some of the dye did not escape into the pericardial cavity, from which absorption is also slow, the percentage excretion in a given time cannot be compared with that in the mammal. In a third experiment (not given here) carried out a year later, an intravenous injection was made and 38 minutes later the urine contained large amounts of phenol red which was definitely more concentrated than in the plasma.

Experiment 1. Lophius III. August 14, 1926.

9:25 Bladder emptied.
9:32 An intraperitoneal injection of 72 mgm. of phenol red (in 12 cc. water) was made.
10:02 Urine, 3.0 cc., no P.R. present.
10:44 Urine, 6.5 cc., faint trace P.R.
Bled. Plasma contains trace P.R.
11:32 Urine, 3.5 cc., contains P.R.
Bled. Urine contains about 4 times as much P.R. as plasma.
12:25 Urine, 4.0 cc., contains P.R.
Bled. Urine contains about 8 times as much P.R. as plasma.
August 15: 11:00 Urine, 700 cc., contains 6.6. mgm. P.R.
August 16. Urine, 50 cc., contains much P.R.

Expt. 2. Lophius V. August 28, 1926.

10:00 Bladder emptied.
10:15 Injected 12 mgm. of P.R. (in 2 cc. saline) into heart.

10:45 Urine, 21 cc., contains 0.43 mgm. per cent P.R.
10:47 Blood drawn. Plasma = 0.30 mgm. per cent P.R.
11:15 Urine, 10.2 cc. contains 3.5 mgm. per cent P.R.
11:20 Blood drawn. Plasma = 0.25 mgm. per cent P.R.
11:50 Urine, 0.8 cc., contains 5.0 mgm. per cent P.R.
11:55 Blood drawn. Plasma = 0.10 mgm. per cent P.R.

Between 11:15 and 11:50 plasma decreased from 0.25 to 0.10 mgm. per cent of phenol red. Average 0.18 mgm. per cent. Urine collected during this period contained 5.0 mgm. per cent. Therefore, phenol red was concentrated 28 times.

Discussion. In the anatomical section of the present report, we have given the evidence which justifies us in considering the kidney of *Lophius* as in the main a tubular organ. However, even if one assumes that all of the pseudoglomeruli have tubular connections and are functional, it is impossible to conceive that the water of the urine could be eliminated entirely by this route. The following calculation will make this clear. We have obtained 700 cc. of urine from a specimen from *Lophius* in the course of 22 hours, an elimination at the rate of 0.53 cc. per minute. In an entire kidney only 78 glomeruli were found or 156 glomeruli in two kidneys. Each glomerulus would, therefore, have to eliminate 3.4 cu. mm. of fluid per minute, a volume that is 800 times the total volume of a glomerular capsule. If one were to assume no tubular excretion, this figure would have to be increased many times to allow for concentration of urinary constituents by the reabsorption of water. It is evident, therefore, that whatever excretory function this kidney possesses must be predominantly tubular in nature.

Although it appears certain that tubular elimination of water and other substances takes place in the kidney of *Lophius*, there is as yet no distinct proof that the process by which the tubules excrete is secretion. In the past, in discussions of the functions of the tubules of the kidney, it seems that tubular excretion and tubular secretion have been regarded as synonymous terms. It is, however, quite possible that excretion might take place across the tubular epithelium and not involve secretion. This necessitates that we have a clearer definition of what we mean by secretion. To those processes occurring in the cells of glands which manufacture some specific product to be eliminated, everyone would apply the term secretion. It must be

remembered, however, that even in the case of these glands, water and other blood constituents may be changed in concentration in their passage through the gland. The elimination of these substances would also be generally regarded as secretion. In the case of the kidney we have the same two types of processes to consider, the first (*e.g.*, synthesis of hippuric acid and its elimination) representing a minor function of the organ, as far as is known at present, and the second the elimination of substances preformed in the blood. Tubular secretion may be defined as the passage of material from blood or lymph through the tubular epithelium to the lumen of the tubule by a process which involves an energy change determined by the activity of the cell; the material may undergo either a change of state or a synthesis or degradation. This definition would be inclusive of a more limited one based on the histological finding of granules or vacuoles in the cells which furnish the product of the secretion, since it is difficult to conceive of granules or vacuoles in the cell being eliminated without involving energy changes determined by the activity of the cell.

It seems, therefore, at present, that our results herein reported concerning the kidney of *Lophius* prove only that tubular excretion of water and other bodies occurs. Whether or not this tubular elimination is accomplished by secretion must be determined by further experiments, which are planned for the near future. Certain considerations which have some bearing on this question may, however, be mentioned. It is evident that the blood pressure in the tubular capillaries must be extremely low because most of the blood reaching the kidney has already passed through two separate capillary beds. The protein content of *Lophius* plasma is fairly high; in one instance it was found to be 3.4 per cent. This would seem to make it doubtful for any filtration pressure to be available for transferring a non-colloidal fluid across the tubule. We have also, in two instances, measured blood pressures in the ventral and dorsal aortae of *Lophius* and found them quite low, but prefer to accumulate more data and perform other types of experiments before discussing further the question of tubular secretion in *Lophius*.

SUMMARY

The kidney of *Lophius piscatorius* has been studied from the standpoint of its structure and function. Serial sections of an entire kidney showed only 78 structures which might be regarded as glomeruli. These structures exhibit certain peculiarities, and some of them at least do not appear to have a functional connection with a tubule. A maceration study indicated that all the tubules observed ended blindly. An estimate of the number of tubules in the serially sectioned kidney gave 150,000 as a minimum or 1 pseudoglomerulus to about 2,000 tubules.

The kidney eliminates urine which may differ markedly in its composition from the blood plasma: some constituents (*e.g.*, magnesium, sulphate, creatine) may be present in more concentrated form, while others (*e.g.*, phosphate) may be in more dilute solution in the urine than in the blood. It seems evident that we have in *Lophius* a predominantly tubular organ, and that we are dealing here with tubular excretion of all substances in the urine.

We are indebted to a "Special Fund for Physiological Research" for part of the expenses of this investigation, and wish to thank Dr. G. B. Wislocki for the microphotographs and for many valuable suggestions.

ADDENDUM

Since the above has been sent in for publication we have had the opportunity at the Marine Biological Laboratory, Woods Hole, to continue our experiments on the kidney of this interesting fish. About thirty pseudo-glomeruli were isolated by teasing from maceration preparations of kidneys which had been previously injected intra-arterially with prussian blue reagents, but in no case has it been possible to demonstrate any tubular connection. Many blind outpouchings such as are described above have, however, been found. We feel, therefore, that the great majority, if not all, of the pseudoglomeruli are not connected with renal tubules. Material has been obtained for a further study of this point by means of carefully prepared serial sections. Additional evidence that the pseudoglomeruli are non-func-

tional is furnished in the experiments mentioned below on the inability of this kidney to excrete ferrocyanide.

Although we have found definitely that a "brush border" occurs on the lumen side of the tubular cells, we cannot at present state whether or not this is true for all the cells of any one tubule or whether a part of the tubule lacks "brush border" as is claimed for the kidney of the Lophobranchs by Verne (1921–22). Material for a further investigation of this matter is at hand.

It has also been found that under some conditions the chloride concentration in the urine may be much lower than its concentration in the blood plasma, which makes the statement in the body of the paper "that the chloride concentration in the urine is always slightly higher than that in the blood" not entirely correct.

The most remarkable of our recent findings is the demonstration of the inability of the kidneys of *Lophius* to excrete either glucose or ferrocyanide when intravascular injections of these substances are made. An injection of 1 gram per kilo of glucose is not followed by glycosuria or any appreciable change in the quantity of urine eliminated. Phlorizin given subcutaneously or intravenously does not produce glycosuria, even if an injection of glucose is given with the phlorizin. An intravenous injection of 0.4 gram per kilo of sodium ferrocyanide is not followed by the appearance of any ferrocyanide in the urine, although the blood plasma contains a large amount.

A complete report on the above experiments and others not mentioned here and their bearing on the question of secretion in this kidney will be published at some future time.

REFERENCES

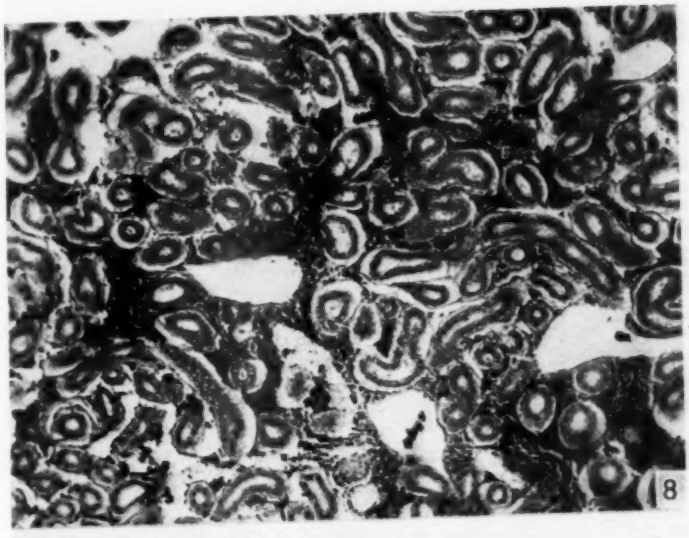
AUDIGÉ, J. 1910. *Arch. de Zool. exp. et gén., 5 me sér.*, iv, 275.
BALFOUR, F. M. 1882. *Quart. Jour. Micros. Sci.*, xxii, 12.
BENEDICT, S. R. 1925. *Jour. Biol. Chem.*, lxiv, 215.
BRIGGS, A. P. 1922. *Ibid.*, liii, 13.
BRIGGS, A. P. 1924. *Ibid.*, lix, 255.
BURIAN, R. 1909. *Ztschr. f. biol. Techn. u. Meth.*, i, 383.
CLARK, E. P., AND J. B. COLLIP. 1925. *Jour. Biol. Chem.*, lxiii, 461.
DAHLGREN, U. 1928. Natural History, xxviii, 18.
DENIS, *N.* 1913. *Jour. Biol. Chem.*, xvi, 389.
DENIS, W. 1922. *Ibid.*, lii, 411.

FOLIN, O. 1904. *Ztschr. f. physiol. Chem.*, xli, 222.
HUBER, G. C. 1911. *Anat. Rec.*, v, 187.
HUOT, A. 1902. *Ann. Sc. Nat. Zool.*, xiv, 197.
KRAMER, B., AND I. GITTLEMAN. 1924. *Jour. Biol. Chem.*, lxii, 353.
KRAMER, B., AND F. F. TISDALL. 1921. *Ibid.*, xlvi, 339.
PETER, K. 1909. Untersuchungen über Bau und Entwickelung der Niere. Jena: Fischer.
ROSENHEIM, O., AND J. C. DRUMMOND. 1914. *Biochem. Jour.*, xiii, 143.
STEWART, S. G. 1927. *Anat. Rec.*, xxxvi, 259.
TRAUT, H. F. 1923. *Contrib. to Embryol., No. 76, Carnegie Inst. of Wash.*, xv, 105.
VAN SLYKE, D. D. 1923. *Jour. Biol. Chem.*, lxiii, 523.
VAN SLYKE, D. D. 1925. *Proc. Soc. Exp. Biol. and Med.*, xxii, 486.
VERNE, J. 1921–22. *Arch. d'Anat. micros.*, xviii, 357.
VINCENT, S. 1898. *Trans. Zool. Soc., London*, xiv, 77.

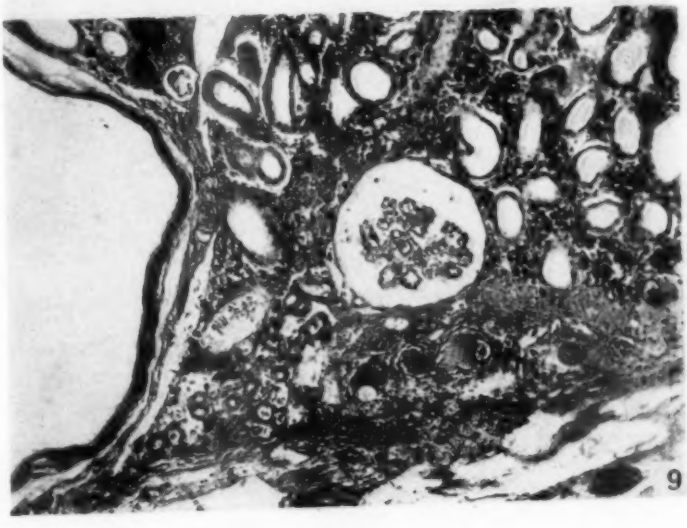
PLATE 1

FIG. 8. Section of kidney completely injected with 50 per cent India ink, to show general arrangement. ×126.

FIG. 9. Thin section of a pseudoglomerulus and sinusoidal tissue. The capillary nature of the tuft is well shown. The large ganglion cells are not a constant finding near the pseudoglomeruli, but have been found in several instances. ×126.



8



9

PLATE 2

FIG. 10. Photograph showing tubular outlet from Bowman's capsule. However, the tubular outpouching ends blindly so far as we can determine. There is an abundance of coagulated fluid. ×370.

FIG. 11. Pseudoglomerulus showing cellular bridges connecting tuft with Bowman's capsule. No tubular outlet could be found in this instance. ×110.

10

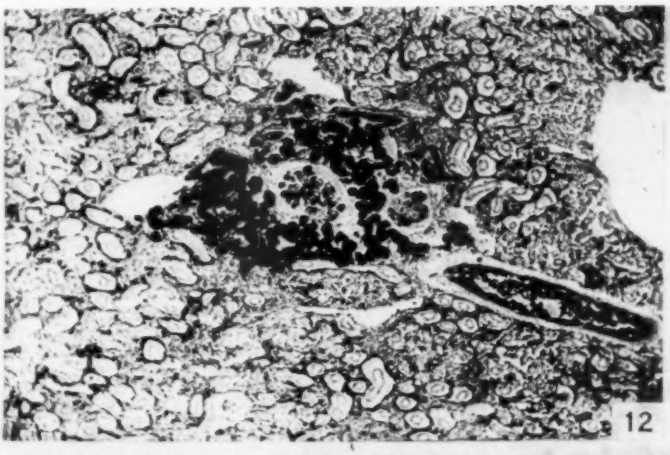
11

PLATE 3

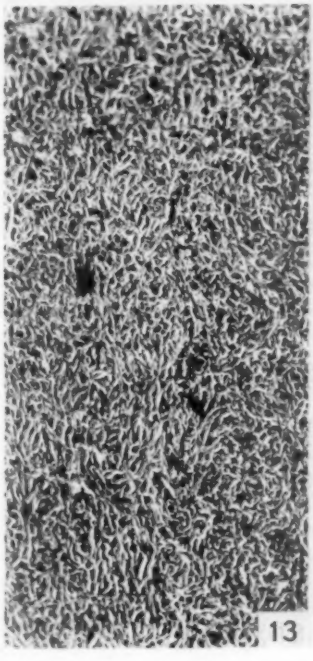
FIG. 12. Thin section of a tuft of sinusoidal tissue containing two pseudoglomeruli, from a completely injected kidney. Note the supplying arterial branch to the right. ×54.

FIG. 13. Thin section of white body. ×126.

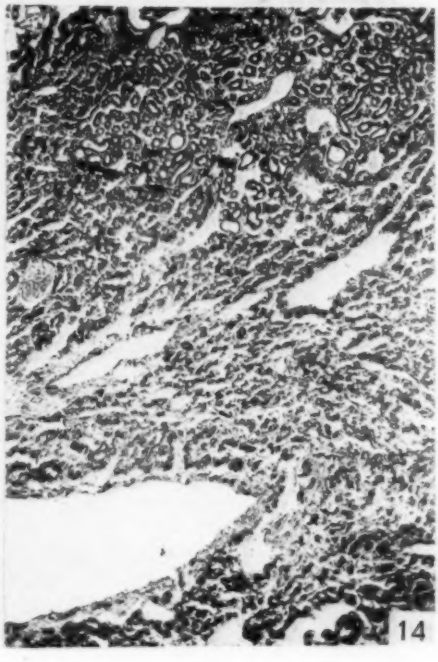
FIG. 14. Mixed interrenal and chromaffin tissue encircling posterior cardinal vein. ×54.



12



13



14

UNIV OF MICH.

THE THRESHOLD FOR ELECTRICAL EXCITATION OF THE MOTOR CORTEX OF ANESTHETIZED MAMMALS[1]

SARAH SHELDON TOWER

From the Henry Phipps Psychiatric Clinic, the Johns Hopkins University, with assistance from the Epilepsy Medical Research Fund

A threshold to any specific form of stimulation is postulated for all excitable tissues. For the electrically excitable portions of the cerebral cortex such a threshold has long been assumed and scattered observations bearing on its value have been reported, largely from Sherrington's school. To the writer's knowledge, however, determinations of the threshold over a period of time have not previously been made on any animal. In a projected study of the influence of changes in intracranial pressure on the electrical excitability of the motor cortex of anesthetized mammals the initial problem therefore presented itself of measuring the threshold in a series of animals over a period of hours to determine both its constancy in the same animal and its comparability from animal to animal.

The experiments which yielded the data for this report were carried out on seventeen cats and seven dogs. Throughout the investigation a uniform procedure was followed; the technique of stimulation only being altered, and with the dogs, the posture. The animals were kept under ether anesthesia, using tracheal cannulation, a T-tube and a Woulfe bottle. Throughout the series of cats a uniform depth of anesthesia was striven for, and to a surprising extent attained,—a level at which all four limbs showed slight extensor rigidity, knee-jerks being present, lid reflexes active and pinna reflexes usually obtainable. A similar state was sought in the dogs but less satisfactorily achieved.

The animals were placed on a rack, the cats with the dorsal surface up and the legs hanging free; the dogs with the dorsal surface down. A clamp held the head rigid. The animals were wrapped in cotton and towels and one or two electric bulbs were used as necessary to

[1] This paper was submitted by the author for the William H. Howell award for 1928 and was selected by the Committee for that award.

maintain the rectal temperature constant within 1°F. Injections were made from a burette through a cannula into the femoral vein of the side on which the skull was trephined. Solutions injected were at body temperature. The subarachnoid space was tapped through the occipito-atlantoid ligament, using a 18-gauge needle. This was connected by rubber tubing to a U manometer of 1 mm. bore with a range of from −100 to +400 mm.; the manometer was filled to the mark 120 mm. with physiological salt solution before being attached to the needle. The skull was opened with a 1 cm. trephine. If correctly placed, the opening thus made sufficed to expose the motor cortex.

Monopolar stimulation of the cortex was used throughout the experiments. As to the technique of stimulation these group into three series. In the first two series, conducted on cats, a spring electrode was used, consisting of a platinum point soldered to a fine steel spring and all enclosed in a glass tube, the end of which was drawn to a diameter of 2 mm. The electrode was mounted on a mechanical stage which, in turn, was attached to the barrel of a microscope. By means of the two screws of the mechanical stage and the microscope adjustment, movement of the electrode in three dimensions was obtained, and the maintenance of the same point and pressure of stimulation throughout an experiment was ensured, though the electrode was removed between stimulations. In one series of experiments the dura was excised and the electrode applied to the arachnoid. It is worthy of note that the spring construction of the electrode and its mechanical manipulation entirely obviated injury to the delicate arachnoid. In another series of experiments pressure exerted on the dura by the electrode forced the dura into contact with the arachnoid and the cortex was stimulated through both membranes. In a third series of cats and in the entire series of dogs the dura was left intact and a small oval glass disc, slightly hollow-ground, through which a platinum point was fused eccentrically, was used as the electrode. When fixed in position with bone wax, this reconstructed the cranium as a closed chamber. The indifferent electrode, a large copper plate, gauze wrapped and soaked in salt solution, was placed upon the shaved skin in the lumbar region in cats and below the umbilicus in dogs where two small incisions had been made.

The selection of the point of stimulation and of the movement to be studied presented an interesting problem. Very quickly it became evident that certain movements not only were elicited at a lower threshold of cortical stimulation than were others and were more constant in their appearance on repeated stimulation of the same point, but that they were also, so to speak, more viable (that is, they survived longer in a deteriorating cortex and maintained a more constant threshold over a period of hours). The most constant and viable movement was found in cats to be flexion of the contralateral elbow. In dogs it was flexion of the contralateral hind-leg with flexion at the ankle, the movement of lowest threshold. The point for stimulation lay in cats 2 to 3 mm. anterior to the cruciate sulcus and 6 to 8 mm. from the midline, in the area B of the motor cortex as described by Weed and Langworthy (1926). In dogs, similarly, it was 1 to 3 mm. anterior to the cruciate sulcus and 8 to 12 mm. from the midline. When the arachnoid was exposed and the spring electrode used for stimulation, movements limited to motion at one joint could be accurately localized. Such fineness of discrimination and localization was approximated in stimulating through the dura with this electrode. On the other hand, from the plate electrode the current was in all probability dispersed over a considerable area of the motor cortex and the threshold obtained was that of the most excitable point affected. The loss of discrimination in movement—toe and ankle movements were often combined with flexion at the more proximal joints—and the stronger currents needed to establish a threshold both attest this spread.

For stimulation the current from one dry cell was sent through a Harvard inductorium. Threshold readings were taken in centimeters' distance of the secondary from the primary coil of the inductorium. In the work on cats the secondary coil was approached to the primary until the first movement resulting from stimulation of the motor cortex appeared. For greater accuracy in the dog experiments the secondary coil was brought up to the primary by 1 mm. stages, and at each a five-second stimulation was allowed by opening the short-circuiting key on the secondary coil. Each determination of threshold noted on the charts is the average of two or three readings of threshold taken at intervals of thirty seconds. Every five minutes for the duration of an experiment, were this two hours or six, records were

made of cerebrospinal fluid pressure and of rectal temperature, of the threshold of the motor cortex and of the character of the movement elicited.

The "control" threshold. Before attempting to study the relationship of the threshold of the motor cortex to variables such as intracranial pressure or brain volume, it was necessary to establish a "control" threshold. This proved feasible in proportion to the skill with which certain disturbing factors were regulated. By far the most important of these was anesthesia. Throughout this series of experiments the greatest care was exercised to maintain a uniform depth of anesthesia,—one at which lid reflexes were active and knee-jerks just present, and in the cats, extensor tone present in all four limbs. This was essential if records at all comparable were to be obtained. As the anesthesia was increased behond this the threshold of the motor cortex rose rapidly to extinction. Under lighter anesthesia, as reflex movements complicated the response to motor cortex stimulation, the threshold became unstable.

The temperature of the motor cortex also seemed influential. When the plate electrode was used, the rectal temperature may be considered to have reflected the temperature of the cortex. Chart IV shows the first hour controls on dogs for which this electrode was used. Here, over a range of 6.4°F., the highest thresholds are found in the two animals with the lowest rectal temperature and the two lowest in the animals with the highest rectal temperature. The comparable records from cats (Chart III) cover, probably, too small a range of temperature, only 2.3°F., to show such influence. With the dura or arachnoid exposed the relationship of cortical temperature to body temperature becomes conjectural. Significantly, however, of the seven experiments in which the spring electrode was used on the intact dura, the four in which between stimulations the dura was irrigated with warm saline yielded the lowest thresholds, and, conversely, the highest thresholds were from those in which dry cotton substituted for irrigation. This is shown in Chart II.

The type of electrode used and its mode of application also conditioned the values obtained for the threshold of the motor cortex. The records taken with the arachnoid exposed (Chart I) exhibit conjointly the lowest thresholds and the greatest variations, variation

both from time to time in the same animal and from animal to animal. When the spring electrode pressed the intact dura into contact with the

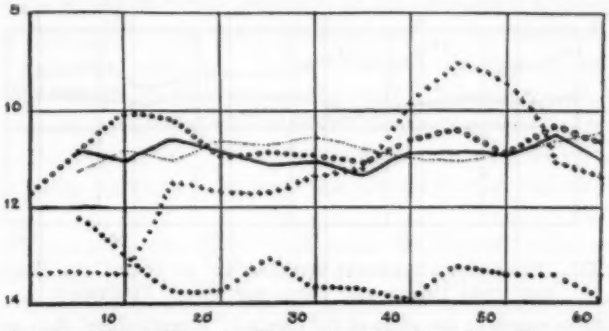


CHART I. FIRST HOUR "CONTROL THRESHOLDS" OF FIVE CATS, TAKEN WITH THE CORTEX EXPOSED AND USING THE SPRING ELECTRODE

Ordinates represent the value of the threshold in centimeters' distance of the primary from the secondary coil of the inductorium. Abscissae represent time in minutes.

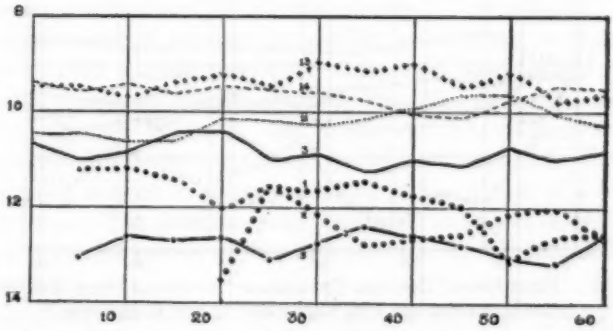


CHART II. FIRST HOUR "CONTROL THRESHOLDS" OF SEVEN CATS, TAKEN WITH THE DURA INTACT AND USING THE SPRING ELECTRODE

Ordinates represent the value of the threshold in centimeters' distance of the primary from the secondary coil of the inductorium. Abscissae represent time in minutes. In nos. 1, 2, 3, and 5 the dura was irrigated, between stimulations, with saline at 38°C. In nos. 11, 13 and 14 dry cotton covered the dura during this interval.

arachnoid, the thresholds (Chart II) were far more constant in the same animal but, on the whole, higher. Mechanical factors operated here.

Though affording protection to the cortex, the dura also interposed a dense connective tissue membrane and a second layer of fluid be-

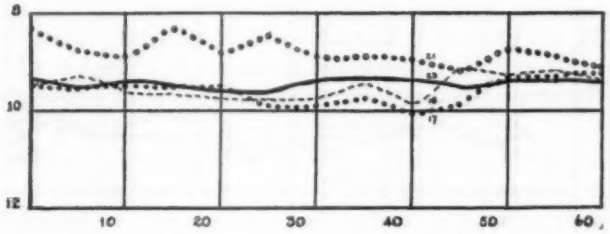


CHART III. FIRST HOUR "CONTROL THRESHOLDS" OF FOUR CATS, TAKEN WITH THE DURA INTACT AND USING THE PLATE ELECTRODE

Ordinates represent the value of the threshold in centimeters' distance of the primary from the secondary coil of the inductorium. Abscissae represent time in minutes. Rectal temperature ranges: No. 17: 100.0°–100.9°F. No. 18: 99.7°–100.3°F. No. 21: 101.7°–101.8°F. No. 23: 99.5°–100.0°F.

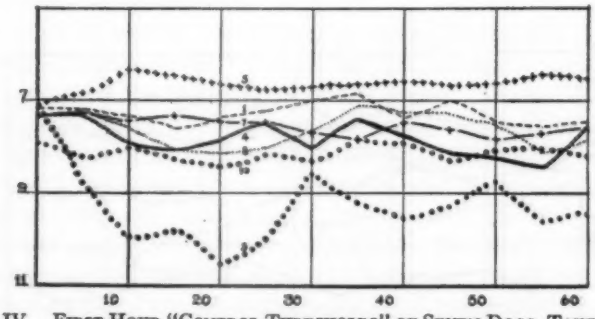


CHART IV. FIRST HOUR "CONTROL THRESHOLDS" OF SEVEN DOGS, TAKEN WITH THE DURA INTACT AND USING THE PLATE ELECTRODE

Ordinates represent the value of the threshold in centimeters' distance of the primary from the secondary coil of the inductorium. Abscissae represent time in minutes. Rectal temperature ranges: No. 1: 96.2°–97.5°F. No. 3: 102.0°–102.6°F. No. 4: 101.6°–102.0°F. No. 5: 98.8°–99.5°F. No. 7: 101.3°–101.8°F. No. 8: 101.1°–101.6°F. No. 10: 102.1°–102.5°F.

tween the electrode and the cortex. When the plate electrode was used to reconstruct the cranium so that cortical temperature and the resistance interposed between the electrode and the cortex were

presumably most constant, the thresholds for motor cortex activity (Chart III) were very stable but higher than those obtained by the use of the spring electrode, although of the same order of magnitude.

The durability of the threshold depended on the conditions under which the cortex was kept. If the arachnoid was exposed, deterioration was continuous almost from the start. When the dura was intact and the spring electrode used, the threshold could be counted on for two hours or more. With the cranium reconstructed by the use of the plate electrode and wax, the threshold was reliable for six hours.

The value of the threshold, measured in centimeters' distance of the primary from the secondary coil of the inductorium is most clearly shown in the charts, together with the range of variation in the individual animal and within the species.

A "control" threshold for the excitability of the motor cortex can therefore be established but its significance remains obscured. Inasmuch as the animals were anesthetized, this "control" threshold bears an as yet undetermined relation to the "normal" threshold of healthy unanesthetized animals, though indubitably it is much higher. Is this threshold which it has proved possible, so to speak, to stabilize in some manner a basic threshold? The rapid deterioration with increasing anesthesia and the apparent instability in lighter anesthesia often suggested as much. Its constancy with uniform technical procedure withing the species, and its different magnitude in cats and dogs again seemed to imply something in the nature of a common least value. The question is one impossible to answer from the data in hand but important for the interpretation of the data now to be presented.

The effect on the "control" threshold of varying the brain volume and the intracranial pressure. The cerebrospinal fluid pressures of the anesthetized animals used in this series of experiments ranged in the cats around 100 mm. of physiological salt solution and in the dogs somewhat higher, more nearly 150 mm.. The cats were old adults, the largest obtainable; the dogs, small adults, frequently almost pups. These pressures were raised, by the intravenous injection of 25 to 200 cc. of distilled water, from two even to five times the initial values, that is, up to 200 to 500 mm. In comparison with intracranial blood

pressure (1500 mm. of salt solution, more or less), these represent moderate increases in intracranial pressure. In no instance were respiratory or vascular disturbances referable to the pressure changes noted. On the other hand, the reduction of intracranial pressure after the injection of 25 to 100 cc. of X 10 Locke's or Ringer's solution was often extreme, from positive pressures of 100 mm. or more to negative pressures of 50 mm. or less. The influence of the intravascular injection of hypertonic and hypotonic solutions on cerebrospinal fluid pressure and on brain bulk has been thoroughly studied by Weed and McKibben (1919).

Inasmuch, however, as these alterations in intracranial pressure were produced by the injection into the vascular system of fluids such as distilled water and concentrated Ringer's or Locke's solution, and were accompanied by marked alterations in brain volume as well as in cerebrospinal fluid pressure, it were well to consider what effect the injection and the changes in brain volume *per se* had on the threshold of the motor cortex. There is an abundance of data on this point. Measurements of threshold made with the dura open exhibited, following injection, the effect of the injection and of the alteration in brain volume alone, for under these conditions the motor cortex was at atmospheric pressure.

Into two cats whose control thresholds were determined as 10.8 and 13.4 cm., respectively (dura excised), 50 and 100 cc. of distilled water were injected over a period of ten minutes. With the smaller quantity, after a temporary fluctuation, the threshold was maintained for twenty minutes. It then began steadily to rise, reaching 9.4 cm. in fifty-five minutes, to be lost at the end of the hour. After the injection of the larger quantity of distilled water, the threshold rose rapidly from the start, to 10.6 cm. at the end of fifty-five minutes, after which no more movements typical of motor cortex excitation were elicited. This deterioration in threshold was obviously concomitant with the protrusion of the brain through the trephine hole and is to be ascribed to vascular disturbance.

Following the injection of concentrated solutions, on the other hand, the rapid shrinking of the brain permitted no such adventitious influence. These observations are, therefore, significant. A cat in which the "control" threshold had been established at about 10.9 cm.

was given an injection of 50 cc. of X 10 Locke's solution over a period of ten minutes. In twenty minutes the threshold had risen to 8.9 cm. where it was approximately maintained for twenty minutes more before excitability was extinguished.

The experiments conducted with the dura intact and using the spring electrode are ambiguous. With moderate increase in brain volume the slight bulging of the dura into the trephine hole no doubt eased the pressure over the motor cortex. When, however, the changes in brain volume and in intracranial pressure were greater, the dura became tense and served then, in all probability, to reconstruct the cranium, equalizing pressure throughout. In any case, however, the cortical blood supply was protected. Four cats whose cerebrospinal fluid pressures ranged from 80 to 130 mm., and whose "control" thresholds had been established at 12.0, 11.0, 9.4 and 12.8 cm., were given injections of 25, 50, 100 and 200 cc. of distilled water. The cerebrospinal fluid pressures rose to 140 mm., 205 mm., 215 mm., and to more than 560 mm. in the several animals. The threshold in the first cat dropped slightly, to 12.7 cm. In the second it was constant while the pressure was high, only to decline when the pressure had returned at the end of one hour to its "control" figure. In the third cat the threshold value was more constant after injection than before, for the two hours during which the pressure rose from 85 mm. to 205 mm. and fell again to 130 mm. In the last cat, while the pressure rose in forty minutes from 130 mm. to exceed 560 mm. (the limit of register), a threshold of 12.8 cm. was maintained until its sudden death from heart failure. These data indicate that increase of brain volume to the extent allowed by the intact dura does not affect appreciably the excitability of the motor cortex of anesthetized animals. Moreover, they support the conclusion that local vascular disturbance alone was responsible for the rapid deterioration of the threshold when, with the dura open, the brain volume was increased.

Because the dura is loose over the cranial vault and could be drawn in as the brain shrank, the pressure on the motor cortex after the injection of concentrated solutions was, in all probability, at all times atmospheric. Two cats whose "control" thresholds had been established at 12.0 and 9.6 cm., cerebrospinal fluid pressures at 130 mm., were each given intravenously, over a period of ten minutes, 50 cc.,

of X 10 Ringer's solution. In both, after temporary rises of pressure to 190 mm. and 210 mm., the pressure dropped rapidly until at the end of forty minutes it was −55 mm. in the first animal and −10 mm. in the second. These negative pressures persisted for the remainder of the hour, after which the pressure was raised in the first cat to +70 mm. by the injection of 50 cc. of distilled water, and in the second rose spontaneously to +15 mm. The threshold in both animals remained almost constant for the ten minutes of injection, and then began rapidly to rise. After forty minutes it stood at 11.0 cm. (C. S. F. pressure −55) in the first cat, and in the second at 8.8 cm. (C. S. F. pressure −10). An hour after injection the threshold was still 11.0 cm. (C. S. F. pressure −55) in the first cat, but down again to 9.0 cm. (C. S. F. pressure +5) in the second. The excitability of the cortex failed to rally with the reestablishment of positive fluid pressures and the threshold rose again in both cats, in the second to extinction at 8.6 cm. fifteen minutes later. These data agree closely with those obtained from the exposed cortex following the injection of concentrated solutions. Hence it appears that, even though the cortex is under a constant pressure (atmospheric), in proportion as the brain is shrunken, the threshold of the motor cortex is increased, *i.e.*, its excitability is diminished, ultimately to be abolished. In contrast, therefore, to the apparent innocuousness of very large quantities of even such a hypotonic medium as distilled water, concentrated solutions such as X 10 Locke's or Ringer's solution in small volume are strongly depressant.

We may consider now the experiments in which intracranial pressure changes were allowed full play because the cranium was closed by the use of the plate electrode and bone wax. Two series of such experiments were completed. In the first, utilizing cats, the threshold was studied only under conditions of normal and increased intracranial pressure. It was not practicable to reduce the pressure below normal because, as the brain shrunk, it receded from the vertically placed electrode and the space between filled with fluid. This was prevented in the second series, for which dogs were used, by placing the animals back down with the head hyperextended so that the electrode over the motor cortex was at the lowest level of the cranium. The application by gravity of the cortex to the dura and thus to the electrode should have been the most constant obtainable.

In three of four successful experiments on cats, the dura was intact; in the fourth there was a small hole in it at the point of stimulation. The first cat maintained a threshold averaging 8.8 to 9.0 cm. for two hours, during which time the pressure rose, following the injection of 100 cc. of distilled water, from 70 to 300 mm. and dropped again to 130 mm. The second and third cats were studied for five hours with three and two injections, respectively, of 100 cc. of distilled water at intervals of an hour. Chart V shows the data on pressure and threshold from the third experiment. The threshold kept a somewhat

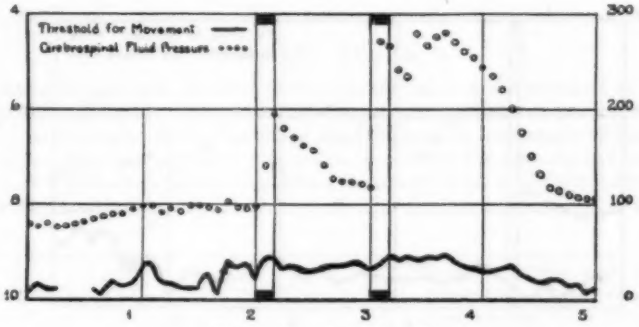


CHART V. CAT NO. 18

Ordinates represent, as noted to the left, the value of the threshold in centimeters' distance of the primary from the secondary coil of the inductorium, and as noted to the right, the cerebrospinal fluid pressure in millimeters of Ringer's solution. Abscissae represent time in hours. During each blocked interval, 100 cc. of distilled water were injected intravenously.

higher level during the whole period covering the two injections of distilled water, but at no time did it respond to a rapid increase in the cerebrospinal fluid pressure, once of more than 200 per cent, with any commensurate rise or fall. The responses of pressure to injection were even greater and more sudden in the second experiment, rising from 85 to 200 mm., again from 100 to 470 mm., and from 80 to 330 mm. in ten minutes. At each of these events the threshold rose slightly, from 9.4 to 9.0 cm., but returned immediately to the "control" value, while the pressure remained high. In the cat with the hole in the dura at the point of stimulation, a "control" threshold of 9.2 cm.

was established and a cerebrospinal fluid pressure of 25 mm. Following a first injection of 100 cc. of distilled water the pressure rose to 116 mm.; the threshold fell to 9.6 cm.; and continued to 10.2 cm. even as the pressure dropped again to 74 mm. On a second similar injection, however, the pressure was raised to 210 mm. without affecting the threshold of 10.1 cm.; then, when the pressure began to decline again, the threshold rose to 9.9 cm. In view of the constancy of the threshold on the second greater increase in intracranial pressure, the first lowering of threshold, or increase in excitability, with rising pres-

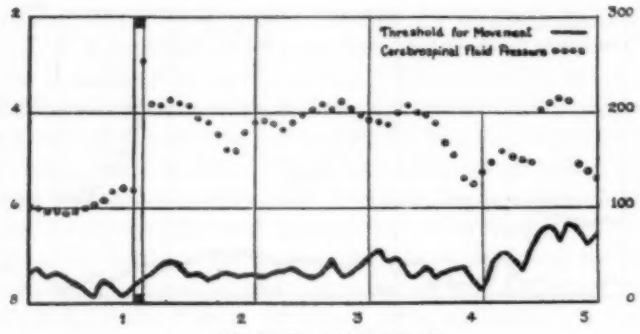


CHART VI. DOG NO. 7

Ordinates represent, as noted to the left, the value of the threshold in centimeters' distance of the primary from the secondary coil of the inductorium, and as noted to the right, the cerebrospinal fluid pressure in millimeters of Ringer's solution. Abscissae represent time in hours. During the blocked interval 50 cc. of distilled water were injected intravenously.

sure can best be ascribed to the closer application of the brain, as it expanded, to the stimulating point, allowed by displacement of fluid at the hole in the dura.

Seven dogs were successfully experimented on for periods of from two to six hours; three received injections of distilled water; one an injection of X 10 Locke's solution; two successive injections of X 10 Locke's solution and distilled water; and one was studied as a control for six hours, with no injection other than 1 cc. of normal saline every ten minutes. The data from these animals are very consistent. Chart VI shows the result of injecting 50 cc. of distilled water. The cere-

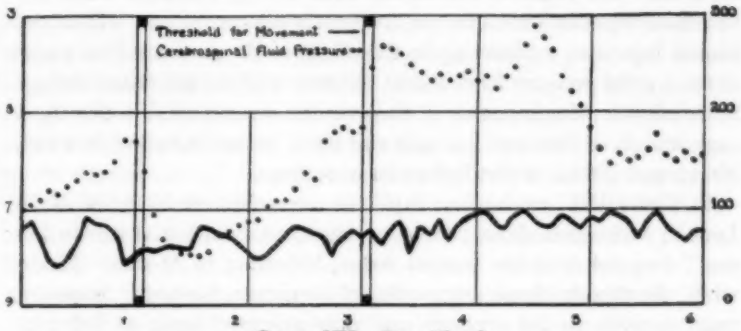


CHART VII. DOG NO. 8

Ordinates represent, as noted to the left, the value of the threshold in centimeters distance of the primary from the secondary coil of the inductorium, and as noted to the right, the cerebrospinal fluid pressure in millimeters of Ringer's solution. Abscissae represent time in hours. During the first blocked interval 25 cc. of X 10 Locke's solution and during the second 50 cc. of distilled water were injected intravenously.

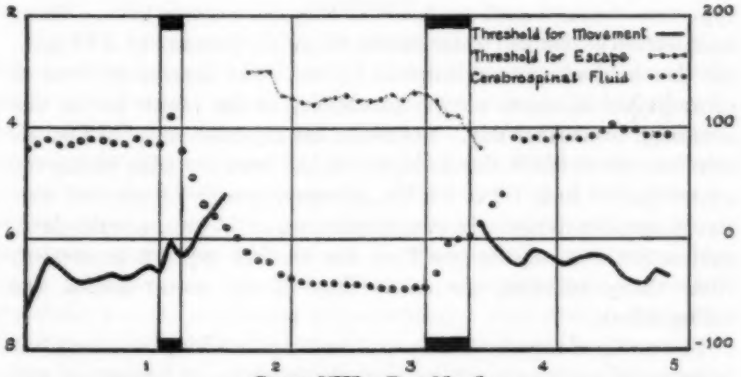


CHART VIII. DOG NO. 5

Ordinates represent, as noted to the left, the value of the threshold in centimeters' distance of the primary from the secondary coil of the inductorium, and as noted to the right, the cerebrospinal fluid pressure in millimeters of Ringer's solution. Abscissae represent time in hours. During the first blocked interval 100 cc. of X 10 Locke's solution were injected intravenously; during the second 200 cc. of distilled water.

brospinal fluid pressure was raised from 120 to 258 mm. while the threshold was but little affected, rising only 0.4 cm. Chart VII, at the second injection, exhibits again the effect of 50 cc. of distilled water in raising the pressure from 180 to 240 mm. with no attendant change in threshold. Confirmation of these results was supplied in two short experiments of two and one-half and three hours' duration in which 300 cc. and 200 cc. of distilled water were given.

In Chart VII, at the first injection, the effect of 25 cc. of X 10 Locke's solution is shown. The pressure, after an initial rise to 184 mm., dropped from the control value, 100 mm., to 52 mm. Meanwhile, the threshold was extraordinarily constant, becoming, however, more variable as the pressure gradually mounted again to 180 mm. Chart VIII demonstrates the disastrous consequences of introducing intravenously 100 cc. of X 10 Locke's solution. From a control value of 85 mm. the pressure fell, with only a slight initial rise, in one hour to −35 mm., and at the end of two hours to −44 mm. The threshold, concomitantly, rose from a "control" of 6.6 cm., to 5.2 cm. in fifteen minutes (pressure 10 mm.) and thereafter no movements of the cortical type were obtained until one hour and fifty-five minutes later. Then, an injection of distilled water having raised the pressure to +15 mm., the threshold was reestablished at 5.7 cm. The depressant effect of concentrated solutions on the excitability of the motor cortex was confirmed in another dog. Following the injection of X 10 Locke's solution, this animal's threshold, which had been dropping during the control period from 7.5 to 8.0 cm., abruptly rose to 7.0 cm. and continued steadily to rise. In twenty minutes, at 6.7 cm., general clonic contraction was superimposed on the cortical type of movement. After thirty minutes, the excitability of the motor cortex was extinguished.

This group of experiments on cats and dogs in which the changes in intracranial pressure resulting from the injection of hypertonic and hypotonic solutions were allowed their full effect, are strikingly comparable with those previously presented, in which pressure changes were to a greater or lesser degree relieved by decompression. No significant differences are apparent. In no experiment did the injection of a hypotonic solution markedly affect the threshold for electrical excitation of the motor cortex. In all, the injection of

hypertonic solutions greatly raised, even abolished this threshold. Apparently, therefore, moderate increase of intracranial pressure is without influence on the excitability of the motor cortex of these anesthetized animals. Furthermore, increase in brain volume to the extent allowed by the intact cranium is inconsequential. On the other hand, reduction in brain volume is exceedingly depressing to the excitability of the motor cortex whether or not accompanied by lowered intracranial pressure. To be sure, two factors must be considered in the causation of this phenomenon, a toxic effect of the material injected as well as the reduction in volume *per se*. The progressive deterioration of excitability concomitant with the shrinking of the brain indicates, however, the latter as the more important, for a toxic effect, as such, would, it seems probable, have been at a maximum immediately after injection. That decreased pressure, as such, was of little consequence was demonstrated by the entirely comparable results with the cranium open and the cortex at atmospheric pressure, and with the cranium closed and pressures as low as −44 mm.

DISCUSSION

The work just presented may be recapitulated in the statment that for the experimental conditions outlined a threshold for electrical excitation of the motor cortex can unquestionably be established and maintained for a period of hours. The value of this threshold is characteristic for each of the two species studied, and fairly constant from animal to animal within the species. The threshold, in anesthetized animals, does not respond measurably to increasing intracranial pressure or to expanding brain volume, whereas, on the contrary, a decrease in brain volume is accompanied by a proportionate raising of the threshold, *i.e.*, by a lowering of excitability. And finally, the relationship of the threshold as studied to the threshold of the motor cortex of unanesthetized animals remains a problem rather for investigation than for conjecture.

These experiments and certain work done previously have a mutual bearing which may merit discussion. Leyton and Sherrington (1917) compared the thresholds for faradic excitation of the motor cortices of a chimpanzee, a macaque and a cat. These animals were arranged for monopolar stimulation of the cortex in series in the secondary circuit

of an inductorium. The threshold excitability for flexion of the contralateral elbow differed in the three animals only one-half centimeters' distance of the primary from the second coil of the inductorium and was highest in the cat. This finding is in contrast to the rather large and constant difference found between cats and dogs in the experiments here reported. Measurements of threshold in a large number of species, in comparable states of anesthesia or unanesthetized, together with analysis of the physical factors conditioning the value of the threshold as measured, will be necessary, however, before one can deduce either that motor cortex tissue has a more or less constant threshold to electrical excitation whenever found; or, on the other hand, that its excitability is a function of the degree of differentiation in the species.

The threshold of the motor cortex hitherto measured or discussed has always been the threshold excitability for a final motor reaction. As motor reactions are more easily detected than inhibition, this is understandable. From clinical experience, however, there is a growing impression that the important rôle of the motor cortex in the total motor mechanism is one of inhibition. In the last century, Bubnoff and Heidenhain (1881) described inhibition of muscular contraction from cortical stimulation in the dog, and Exner later (1892) for the rabbit. Sherrington (1894, 1905) showed that in the eye muscles of monkeys reciprocal inhibition was an accompaniment of motor reactions of cortical origin whether artificially or voluntarily excited. And that the same held for the response of skeletal muscle to cortical stimulation was demonstrated by Herring and Sherrington (1897). Recently, by electrical stimulation of the motor cortex, Muncie and Schneider (1928) produced generalized convulsions in cats which had received non-convulsant doses of oil of wormwood, and then inhibited the clonic convulsive movements in the contralateral limbs by again stimulating one motor cortex. A somewhat similar response was noted repeatedly in the course of the author's experiments. In the stage of anesthesia sought, extensor tone was always present in the limbs of the cats and often in the dogs. Again and again, as the current strength was being increased to determine the threshold for motor response, this extensor tone was inhibited at a threshold from 0.2 to 0.8 cm. lower than the threshold for muscular contraction. Further

studies of this finding are planned. Sherrington noted (Integrative Action: p. 291) that "the usual effect of the cortex on the extensors is inhibitory," but that inhibition has a lower threshold than contraction in the same muscle group had not so far been demonstrated. It is, certainly, an interesting experimental basis for the clinical inference that the normal function of the motor cortex is largely inhibitory.

This research was originally designed to determine by actual measurement the influence on the excitability of the motor cortex of moderate changes in intracranial pressure. The electrical excitability was selected as most easily subjected to exact measurement. Elsberg and Pike (1926) had the same end in view when they tested the susceptibility of unanesthetized cats to convulsions produced by oil of wormwood and at the same time altered the intracranial pressure by the intravenous injection of hypertonic and hypotonic solutions. They found that by increasing the intracranial pressure the convulsive dose of the drug could be reduced to about 60 per cent of the dose for a normal animal, whereas when the intracranial pressure was lowered the dose required was proportionately larger. The failure to show, in the experiments reported in this paper, commensurate alteration in the excitability of the motor cortex accompanying changes in intracranial pressure is probably to be ascribed to the anesthetized condition of the animals. By reducing the excitability of the motor cortex to what appeared to be a somewhat stable least value, it seems not improbable that the sensitivity of the threshold to a variable such as intracranial pressure became less than the technique used could detect; or that the intracranial pressure is one among certainly many factors influencing the activity of the normal cortex to which this basic excitability is totally unresponsive. One half of Elsberg and Pike's work, to be sure, was reproduced, the depression of excitability from the injection of hypertonic solutions, although a more direct effect of the solution, probably on brain volume, not on lowered intracranial pressure, was held responsible for the result.

Three factors, only, were found to affect this stabilized threshold, exclusive, of course, of the state of anesthesia which determined it. These were the physical condition of the cortex, the temperature of the

cortex and reduction in brain volume. The two former so intimately condition the functioning of all living tissue as to need no discussion. The latter seemed to exert a specific proportionate depressing action. That irritability was by no procedure heightened, either by expanding the brain or by increasing the intracranial pressure, suggests that this basic excitability is isolated from all augmenting influences. In this condition no convulsions,—nothing indicative of spread of excitation within the cortex, were ever elicited. With exceedingly strong currents, to be sure, muscular spasms were produced, but any portion of the surface of the brain yielded these, and even after decerebration, if the brain anterior to the cut was left *in situ* and stimulated. The experiments conveyed the impression that one was dealing with excitation of the cell group most immediately affected by the stimulating current and a response in which all such elements as facilitation, reinforcement, whatever might be contributed by spread of excitation within the cortex, were eliminated; in short, with activation limited to the direct descending motor path, exhibited under the best conditions as inhibition followed by contraction, under less favorable conditions as the motor response alone.

The possible rôle of intracranial pressure changes acting on cortical excitability in the precipitation of epileptic attacks challenges consideration. A number of factors, to be sure, must operate in the genesis of epilepsy. An hereditary predisposition is well attested. The frequent association of epilepsy with mental deficiency has been put beyond doubt by statistics from numerous institutions for the feeble-minded and insane. An increase of fluid over the surface of the brain was early and repeatedly observed in epileptics, especially at operation. And numerous lesions, both proliferative and atrophic, have been described in autopsy material. The rôle of focal lesions in the production of Jacksonian fits seems amply certified. On the other hand, epilepsy occurring without demonstrable organic lesions, psychogenic epilepsy, is a frequently reported and much discussed entity. On the physiological side, Cushing (1909) casually noted: "it has seemed to me probable, from other experiences in the past, that the cortex in cases of epilepsy is more excitable than the cortex of non-epileptics."

These, however, are all factors which operate to produce a general

susceptibility to convulsions, and in no way determine the occurrence of the specific attack. Much literature has dealt with the problem of how, from susceptibility, the seizure results. It is entirely possible that the process differs from case to case. For one the precipitant is, perhaps, a vascular disturbance, vasoconstriction or venous stasis, for another, autointoxication, for a third, increased intracranial pressure. Leriche and Wertheimer (1925) concluded, from the study of eighteen cases of Jacksonian epilepsy, fifteen following war wounds, that the basic lesion is a cortical neuroglial sclerosis but that changes in cerebrospinal fluid pressure are the immediate cause of the convulsions. By using a hypertonic solution (10 per cent glucose) and a hypotonic solution (distilled water), as indicated by the cerebrospinal fluid pressure in lumbar puncture, some of these patients were freed of attacks for years. Before this, Ebaugh and Stevenson (1920) had made an intensive study of a trephined epileptic and found that his attacks were associated with a rise in intracranial pressure unaccounted for by activity. In this man, hypotonic and hypertonic solutions had the same influence on intracranial pressure found for the cat by Weed and McKibben (1919). At the height of the rise in intracranial pressure produced by drinking 4000 cc. of water, the patient complained of the symptoms which preceded his spontaneous attacks and once an attack of grand mal was so precipitated. The work of Elsberg and Pike (1926), previously discussed, would indicate that increased intracranial pressure could play the rôle suspected for it. And the depression of cortical excitability reported by the author, after the administration of hypertonic solution provides a basis for one half of Leriche and Wertheimer's therapeutic results. Nevertheless, the fact that a rise of pressure is concomitant with an epileptic seizure is not adequate evidence that it is the cause of the seizure. Indeed, when so many factors are interdependent, as are the cerebrospinal fluid pressure and brain bulk, tissue nutrition and the blood flow, in controlling the function of the brain, to say that a certain event in function is dependent on this or that factor usually means no more than that fortuitously, or through the observer's directed interest, or as the result of the methods used, the event and the factor have come concomitantly under observation.

SUMMARY

The threshold for electrical excitation has been determined for the motor cortex of anesthetized animals under constant conditions of anesthesia and body temperature and with routine experimental procedure. The value of this threshold was characteristic for each of the two species used, cat and dog.

The effect on this threshold of varying the intracranial pressure by the intravenous injection of hypertonic and hypotonic solutions has been studied and found negligible. Moreover, the increase in brain volume resulting from the injection of hypotonic fluids had no measurable effect on the excitability of the motor cortex. The decrease in brain volume, following the injection of hypertonic solutions, was, on the contrary, accompanied by a proportionate raising of the threshold, or lowering of the excitability of the motor cortex.

The reaction of lowest threshold obtained from the motor cortex was inhibition of extensor tone.

The general significance of these results is considered, together with their relation to the precipitation of epileptic attacks and their control.

REFERENCES

BUBNOFF, N., UND HEIDENHAIN, R.: 1881. *Pflüger's Arch.*, xxiv, 137.

CUSHING, H.: 1909. *Brain*, xxxii, 44.

EBAUGH, F. G., AND STEVENSON, G. S.: 1920. *Johns Hopkins Hosp. Bull.*, xxxi, 440.

ELSBERG, C. A., AND PIKE, F. H.: 1926. *Am. Jour. Physiol.*, lxxvi, 592.

EXNER, S.: 1892. *Pflüger's Arch.*, xxviii, 487.

HERRING, H. E., UND SHERRINGTON, C. S.: 1897. *Pflüger's Arch.*, lxviii, 222.

LERICHE, R., AND WERTHEIMER, P.: 1925. *Revue de chirurg.*, lxiii, 641.

LEYTON, A. S. F., AND SHERRINGTON, C. S.: 1917. *Quart. Jour. Exp. Physiol.*, xi, 135.

MUNCIE, W. S., AND SCHNEIDER, A. J.: 1928. *Johns Hopkins Hosp. Bull.*, xlii, 77.

SHERRINGTON, C. S.: 1894. *Jour. Physiol.*, xvii, 27; 1905. *Proc. Roy. Soc., Lond.*, lxxvi, 161, 269.

WEED, L. H., AND LANGWORTHY, O. R.: 1926. *Contrib. to Embryol., Carnegie Inst. Wash.*, Pub. 362, xix, 89.

WEED, L. H., AND MCKIBBEN, P. S.: 1919. *Am. Jour. Physiol.*, xlviii, 512, 531.

MATERIAL FROM LYMPH NODES. III[1,2]

Gonococci from Lymphoid Tissue in a Case of Chronic Infectious Arthritis

CLAUDE E. FORKNER, M.D.

From the Medical Clinic of the Johns Hopkins Hospital

The accounts in the literature of cultures from benign, hyperplastic lymph nodes are very few. Rosenow (1), in 1914, reported the bacteriological findings in cultures from lymph nodes in fifty-four cases of chronic arthritis. In one of these he found gonococci. Full data concerning this case are lacking.

Billings, Coleman, and Hibbs (2), in 1922, made a similar study in twenty-eight cases of chronic infectious arthritis. In no instance were gonococci isolated. Several reports in the literature record the search in benign, hyperplastic lymph nodes for the etiological agents of tropical diseases and of syphilis, but in none of these were gonococci obtained. The following case from the Medical Wards of the Johns Hopkins Hospital may, therefore, be of interest.

CASE REPORT

P. L., male, aged 51, married. Admitted December 15, 1926. *Diagnosis:* Chronic infectious arthritis.

Complaint: Pain and swelling of the joints, especially of the knees, shoulders, and hands.

Past history: General health fair. Frequent severe headaches. No indigestion. Eight years before admission he had had a Neisser infection which subsided, but later he had been reinfected. At that time he had a sore heel for a few days which entirely cleared up. No urethritis or prostatitis for last 7½ years. Constipation for 15 years. Nervous breakdown 15 years and again one year before admission. Loss of 28 pounds in weight in the last 6 months.

Present illness: Onset rather abrupt 8 months ago with pain through the arches of the feet. After 2 months this spread to the left knee, left hip, left

[1] Received for publication July 3, 1928.

[2] The first two papers of this series of studies on lymph nodes were published in the *Archives of Internal Medicine*, 1927, xl, 532 and 647.

hand, and practically all the joints. Three months ago the right temporomandibular joint became affected. There was considerable pain, swelling, and stiffness in the joints.

Physical examination: Somewhat undernourished, middle-aged man with an extremely nervous temperament. Moderate general enlargement of lymph nodes. Tonsils: Scarred, embedded, red, and show a few whitish areas, indicating a chronic tonsillar infection; culture gives a beta-hemolytic streptococcus. Sinuses: Old infection of left antrum. X-ray reported clear. Teeth: None remaining. Heart and lungs normal. Abdomen negative. Spleen not palpable. Prostate very slightly enlarged with a few more pus cells than normal. No gonococci found. Joints: Knees, ankles and hands swollen, tender; some limitation of motion and crepitus; hips, elbows, and shoulders show tenderness, crepitus, and limitation of motion; spine and jaws tender, with limitation of motion.

Laboratory examinations: Blood: R.B.C. 3,400,000; Hgb. 75 per cent (Sahli); W.B.C. 7,800; PMN 72 per cent. Wassermann negative. Urine negative. X-rays: left foot showed a slight infectious arthritis. Lymph-node culture: Lymph-node in left axilla punctured (3, 4) January 8, 1927. Gonococci recovered in pure culture. Material withdrawn showed an increased number of neutrophiles in the node and evidence of benign hyperplasia. There was no necrosis. Aspiration of left knee January 14, 1927: Fluid small in amount and yellow in color. Gonococci recovered in pure culture. Synovial fluid cell count: 3,240 per cubic millimeter. Differential: neutrophiles 87 per cent; lymphocytes 5 per cent; monocytes 8 per cent. Aspiration of left knee February 23, March 10, and March 27. Gonococci recovered in pure culture on each of these dates.

This case is reported in order to call attention to the fact, not generally recognized, that gonococci may be the etiological agents in a small, but definite percentage of patients with chronic infectious arthritis. Furthermore, the case shows that the lymph nodes may harbor these organisms over a long period of time and may act as secondary foci of infection. There was no necrosis of the lymph nodes in this case. This is significant because there is no case report in the literature of cultures of gonococci from lymph nodes which are not themselves necrotic.

The organisms were cultured on freshly prepared ascitic dextrose agar. They failed to grow on simple media. They were Gram-negative diplococci presenting the typical morphological and cultural characteristics of gonococci. A vaccine was made with which the patient was treated. Marked clinical improvement followed this procedure. In order to ensure growth it is important that the media be prepared within a short time of the inoculation.

REFERENCES

(1) ROSENOW, E. C.: The newer bacteriology of various infections as determined by specific methods. *Jour. Amer. Med. Assn.*, **63**, 905, 1914.

(2) BILLINGS, F., COLEMAN, G. H., AND W. G. HIBBS: Chronic infectious arthritis. Statistical report with end-results. *Jour. Amer. Med. Assn.*, **78**, 109, 1922.

(3) FORKNER, C. E.: Material from lymph nodes of man. Method to obtain material by puncture of lymph nodes for study with supravital and fixed stains. *Arch. Int. Med.*, 1927, xl, 532.

(4) *Idem:* Material from lymph nodes of man. Studies on living and fixed cells withdrawn from lymph nodes of man. *Arch. Int. Med.*, 1927, xl, 647.